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First record of *Erysiphe howeana* from China

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Abstract — *Erysiphe howeana* (*Erysiphe* sect. *Erysiphe*) was identified on *Oenothera biennis*. This is the first record of this powdery mildew from China. The Chinese specimen is described, illustrated, and discussed, and its identity is confirmed by means of molecular methods.

Key words — common evening primrose, morphology, molecular phylogeny, distribution data, Asia

Introduction

In the autumn of 2014, *Oenothera biennis* (*Onagraceae*) heavily infected by a powdery mildew was found in Shaanxi, China. Based on the morphological characteristics of the asexual morph, it was classified as *Erysiphe* sp. Molecular analyses were applied to resolve the species identity. The internal transcribed spacer (ITS) and 28S rDNA regions were sequenced and compared with sequences in corresponding depositories. Based on sequence analysis of the rDNA ITS region, this species has been identified as *Erysiphe howeana*.

This species had not previously been reported from China (Zheng & Yu 1987, Wu & Wu 1991, Chen 1993, Wang et al. 2002, Liu 2010), and our collection constitutes the first Chinese record.

Materials & methods

Living leaves of *Oenothera biennis* bearing the anamorph of a powdery mildew were collected in October 2014 in the Qinling Mountains within Shangbahe Forest Park in China. A herbarium specimen was deposited in the Mycological Herbarium of Forestry College, Northwest A & F University, Yangling, Shaanxi Province, China (HMNWAFU - CF).

A sample was mounted in distilled water and examined using light microscopy (Olympus, CX31RTSF, Japan). A scanning electron microscope (JEOL, JSM-6360LV) was used to observe the anamorph ultrastructure, particularly the surface features of conidia (according to Cook et al. 1997) and hyphal appressoria of this fungus.

Genomic DNA was extracted from mycelium and the asexual morph using Chelex-100 (Walsh et al. 1991, Hirata & Takamatsu 1996). The ITS region of the nuclear rDNA (including 5.8S) and 28S rDNA sequences with domains D1 and D2 were amplified via polymerase chain reaction (PCR); PM5 and PM6 (Takamatsu & Kano 2001) were used to amplify the ITS region, while LSU1 and LSU2 (Scholin et al. 1999) were used to amplify the 28S rDNA sequence.

The PCR assays were conducted in a 50 μL final volume (Hirata & Takamatsu 1996) containing 27 μL of 2× BoisTaq PCR MasterMix, 1 μL of each of primer, 1 μL of the extracted DNA, and 20 μL of ddH2O (Hirata & Takamatsu 1996). Thermal cycling in a PTC-200 thermal cycler (BioRad) comprised an initial denaturation step at 95°C for 5 min, 35 cycles of 94°C for 1 min + 60°C for 1 min + 72°C for 1 min, and a final elongation step at 72°C for 8 min. A negative control for each set of reactions replaced template DNA with ddH_2O. The PCR products were separated by electrophoresis on a 2% agarose gel in TAE buffer and purified using the Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer's instructions. The purified DNA products were ligated into the pMD18-T vector (Takara) and transformed into *E. coli* DH5α cells. The cloned fragments were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

All DNA sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignments were adjusted following Nei & Kumar (2000). All positions containing gaps or missing data were eliminated from the dataset. Cladistic trees were constructed using the neighbor-joining method with the Kimura 2-parameter substitution model in MEGA 4.0 (Tamura et al. 2007). Branch robustness was assessed by bootstrap analysis with 1000 replicates.

Taxonomy

Erysiphe howeana U. Braun, Mycotaxon 14: 373, 1982. PLATE 1

SPECIMEN EXAMINED: **CHINA, SHAANXI,** Qinling Mountains, Shangbahe Forest Park, 33°21′42″N 108°27′07″E, alt. 1472 m, on living leaves of *Oenothera biennis* L., Oct. 2014, L.C. Bai (HMNWAFU-CF 2014001; GenBank KP247216, KP247417).

Mycelium mainly foliicolous, amphigenous, also on stems and fruits, effuse or in irregular patches, often covering the entire leaf surface, white, persistent. Hyphal appressoria lobed, solitary or in opposite pairs; hyphae smooth or almost smooth, 3–7 μm wide, colorless. Conidiophores arising from the upper surface of mother cells, erect, straight or somewhat curved, foot-cells cylindrical, about 23–42 × 6–11 μm, followed by 1–2 shorter cells, forming conidia singly. Conidia ellipsoid-ovoid, doliiform or subcylindrical, 22–33 × 10–17 μm, with a length/width ratio of 1.3–2.2.

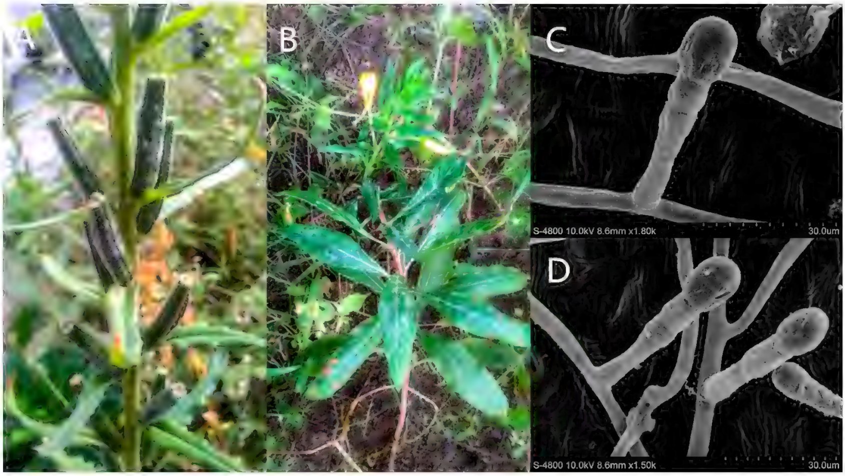

PLATE 1. *Erysiphe howeana* (HMNWAFU-CF 2014001): A & B. *Oenothera biennis* infected by a powdery mildew; C & D. Conidiophore, conidia, and hyphae.

Phylogeny

28S ANALYSIS PLATE 2

The 28S rDNA sequence comprising 597 total characters was deposited in GenBank under accession number KP247216. The sequence was aligned with 15 sequences representing the five tribes of *Erysiphaceae*. The 28S rDNA phylogenetic tree places the *Erysiphe howeana* sequence in one clade (bootstrap value = 98%) where it clusters with *E. paeoniae* R.Y. Zheng & G.Q. Chen, clearly supporting its placement in the tribe *Erysipheae*. *Byssoascus striatosporus* (G.L. Barron & C. Booth) Arx was used as outgroup.

ITS ANALYSIS PLATE 3

The ITS rDNA sequence comprising 398 total characters was deposited in GenBank under accession number KP247217. The sequence was aligned with 16 sequences representing *Erysiphe* R. Hedw. ex DC. (emend. Braun & Takamatsu 2000). *Erysiphe adunca* (Wallr.) Fr. var. *adunca* was used as outgroup. The ITS phylogenetic tree placed the Chinese specimen and *Erysiphe howeana* (GenBank AF011301, based on North American material) in a strongly supported clade with 100% bootstrap support, indicating that these two sequences represent the same species.

Discussion

The teleomorph of *Erysiphe howeana* was not found in the Chinese collection. Powdery mildew on *Oenothera* spp., including worldwide collections of

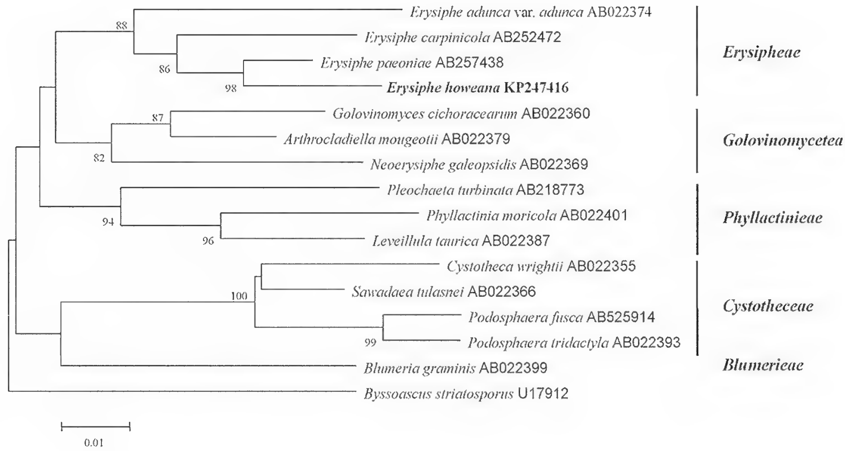
Erysiphe adunca var. adunca AB022374
88
Erysiphe carpinicola AB252472
86
Erysiphe paeoniae AB257438
98
Erysiphe howeana KP247416
87
Golovinomyces cichoracearum AB022360
Arthrocladiella mougeotii AB022379
82
Neoerysiphe galeopsidis AB022369
Pleochaeta turbinata AB218773
94
Phyllactinia moricola AB022401
96
Leveillula taurica AB022387
Cystotheca wrightii AB022355
100
Sawadaea tulasnei AB022366
Podosphaera fusca AB525914
99
Podosphaera tridactyla AB022393
Blumeria graminis AB022399
Byssoascus striatosporus U17912
Erysipheae
Golovinomycetea
Phyllactinieae
Cystotheceae
Blumerieae
0.01

Plate 2. Neighbor-joining tree derived from 28S rDNA sequences from 15 taxa of *Erysiphaceae* with *Byssoascus striatosporus* as outgroup. The bar indicates a distance of 0.01.

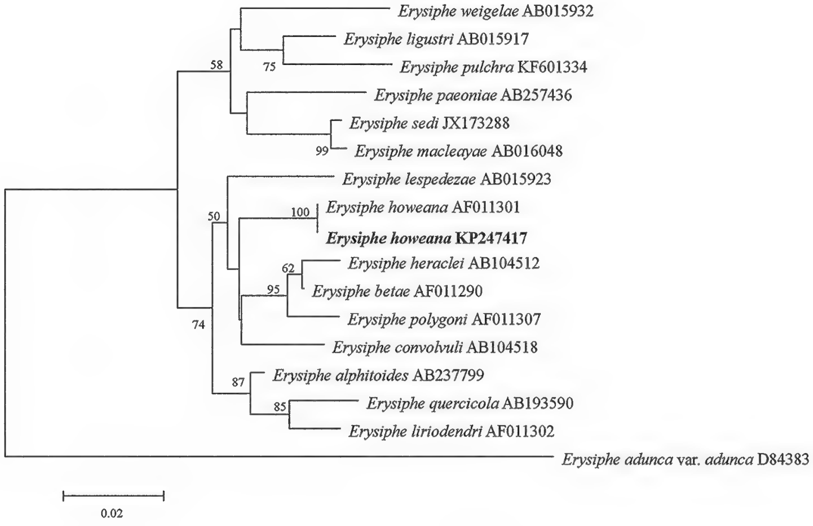
Erysiphe weigelae AB015932
Erysiphe ligustri AB015917
58
75
Erysiphe pulchra KF601334
Erysiphe paeoniae AB257436
Erysiphe sedi JX173288
99
Erysiphe macleayae AB016048
Erysiphe lespedezae AB015923
100
Erysiphe howeana AF011301
50
Erysiphe howeana KP247417
62
Erysiphe heraclei AB104512
95
Erysiphe betae AF011290
Erysiphe polygoni AF011307
74
Erysiphe convolvuli AB104518
87
Erysiphe alphitoides AB237799
85
Erysiphe quercicola AB193590
Erysiphe liriodendri AF011302
Erysiphe adunca var. adunca D84383
0.02

Plate 3. Neighbor-joining tree derived from ITS1 + 5.8S rDNA + ITS2 sequences from 16 *Erysiphe* taxa with *Erysiphe adunca* var. *adunca* as outgroup. The bar indicates a distance of 0.02.

Pseudoidium-like asexual morphs, has been generally referred to *E. howeana*, originally described from North America (Braun 1987, Braun & Cook 2012). However, Bereczky et al. (2015), who sequenced powdery mildew on *Oenothera* spp. from different parts of the world, found surprising results. Two different species with morphologically minimally distinguishable anamorphs have been identified on *Oenothera* spp., namely the common *E. howeana* and the oak powdery mildew *E. alphitoides* (Griffon & Maubl.) U. Braun & S. Takam., which may occur on evening-primrose. Therefore, identification of *Oenothera* powdery mildew based on the oidium alone is barely possible. Thus molecular methods (ITS rDNA sequences) were applied to confirm the identity of the Shaanxi specimen as *E. howeana*. This species has been reported from South Africa, North America, and Europe, but not previously from Asia. This is the first record of *E. howeana* for China.

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